

1256429

TO: ANDREW TOWNSEND, THEODORE M. KENNEDY, SCHAUMBURG, ILLINOIS, UNITED STATES

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

December 03, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM
THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK
OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE.

APPLICATION NUMBER: 60/514,694

FILING DATE: *October 27, 2003*

RELATED PCT APPLICATION NUMBER: PCT/US04/34667

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office



Please type a plus sign (+) inside this box →

PTO/SB/18 (5-03)

Approved for use through 04/30/2003. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

U.S.P.O.
60/514694
22154
102703

INVENTOR(S)		
Given Name (first and middle [if any]) John Joseph	Family Name or Surname Partridge	Residence (City and either State or Foreign Country) Durham, North Carolina
<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto		
TITLE OF THE INVENTION (280 characters max) Enzyme-Catalyzed Dynamic Kinetic Resolution Process for Preparing (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol and Pharmaceutically Acceptable Salts		
Direct all correspondence to: CORRESPONDENCE ADDRESS		
<input checked="" type="checkbox"/> Customer Number <input data-bbox="383 749 791 792" type="text" value="23347"/> →		<input type="checkbox"/> Place Customer Number Bar Code Label here
OR <input data-bbox="440 813 718 834" type="text" value="Type Customer Number here"/>		
<input type="checkbox"/> Firm or Individual Name		
Address		
Address		
City	State	ZIP
Country	Telephone	Fax
ENCLOSED APPLICATION PARTS (check all that apply)		
<input checked="" type="checkbox"/> Specification Number of Pages <input data-bbox="579 1077 693 1108" type="text" value="30"/>	<input type="checkbox"/> CD(s), Number <input data-bbox="1085 1077 1183 1108" type="text"/>	
<input type="checkbox"/> Drawing(s) Number of Sheets <input data-bbox="579 1129 693 1161" type="text"/>	<input type="checkbox"/> Other (specify) <input data-bbox="1085 1129 1403 1161" type="text"/>	
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76		
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)		
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees	FILING FEE AMOUNT (\$)	
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number <input data-bbox="791 1320 987 1351" type="text" value="07-1392"/>	<input data-bbox="1150 1320 1297 1351" type="text" value="\$160.00"/>	
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.		
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.		
<input checked="" type="checkbox"/> No.		
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____		

Respectfully submitted,

Date 23 October 2003

SIGNATURE Bonnie L. Deppenbrock

REGISTRATION NO. 28,209

TYPED or PRINTED NAME Bonnie L. Deppenbrock

(if appropriate) PR60552P

TELEPHONE 919-483-1577

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10)				Docket No. PR60552P
Applicant(s): John Joseph Partridge				
Serial No. To be Assigned	Filing Date	Examiner	Group Art Unit	
Invention: Enzyme-Catalyzed Dynamic Kinetic Resolution Process for Preparing (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol and Pharmaceutically Acceptable Salts				
<p>I hereby certify that this <u>Provisional Application</u> <small>(Identify type of correspondence)</small> is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: Director of the United States Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450 on <u>10/27/03</u> <small>(Date)</small></p> <p style="text-align: center;"><u>Allyson K. Jacobs</u> <small>(Typed or Printed Name of Person Mailing Correspondence)</small> <u>Allyson K. Jacobs</u> <small>(Signature of Person Mailing Correspondence)</small></p> <p style="text-align: center;"><u>EV330916765US</u> <small>(Express Mail Number)</small></p> <p>Note: Each paper must have its own certificate of mailing.</p>				

**Enzyme-Catalyzed Dynamic Kinetic Resolution Process for Preparing
(+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol and
Pharmaceutically Acceptable Salts**

5 **BACKGROUND OF THE INVENTION**

1. **Field of the Invention**

The present invention relates to a process for making (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol and pharmaceutically acceptable salts such as the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt by an enzyme-catalyzed dynamic kinetic resolution of the racemate (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol.

2. **Description of the Prior Art**

(+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol and pharmaceutically acceptable salts and solvates thereof, and pharmaceutical compositions comprising the same are used in treating numerous diseases or disorders such as depression, attention deficit hyperactivity disorder (ADHD), obesity, migraine, pain, sexual dysfunction, Parkinson's disease, Alzheimer's disease, or addiction to cocaine or nicotine-containing (especially tobacco) products.

Several literature references describe the preparation of either the (+)-(2S, 3S) or (-)-(2R, 3R)-enantiomers from (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol. For instance, reference is made to U.S. Patent No. 6,342,496 B1, issued to Jerussi et al. on January 29, 2002, U.S. Patent No. 6,337,328 B1, issued to Fang et al. on January 8, 2002, U.S. Published Applications 2002/0052340A1, and 2002/0052341A1, as well as WO 01/62257 A2. Reference is also made to pending U.S. Application No. 10/147,588; to U.S. Patent No. 6,274,579; and to U.S. Patent No. 6,391,875.

U.S. Patent No. 6,337,328, U.S. Published Application 2002/0052341A1, WO 01/62257 A2, and U.S. Application No. 10/147,588 refer to a chiral acid resolution method for preparing (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol from the racemate (+/-)-(2R*,

3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol. However, the method described in each of these references differs from the present invention in both procedure and result. These references relate to chemical resolutions of the racemate, while the present invention involves the enzyme-catalyzed 5 dynamic kinetic resolution of the racemate. In the simple chemical resolution of the racemate, these references must isolate the desired diastereomeric morpholinol from a mixture of diastereomeric salts. The maximum yield of the desired diastereomer can therefore be at most 50%.

In general, most chemical resolutions of a racemic material, such as 10 Fang et al and Jerussi et al, produce the desired enantiomer or mirror image diastereoisomer in a maximum theoretical yield of 50%. The undesired enantiomer or mirror image diastereoisomer is discarded as waste in the mother liquor.

There are a few instances in which a maximum theoretical yield of 15 100% of a particular specific enantiomer can be obtained by a chiral enzymatic reaction on a pro-chiral substrate. This process is sometimes termed "an enzymatic hydrolytic desymmetrization". Such a chiral enzymatic reaction on a pro-chiral substrate for five closely related compounds is set forth in "Enantioselective Hydrolysis of cis-3, 5-Diacetoxycyclopentene: 1R, 20 4S-(+)-4-Hydroxy-2-cyclopentenyl Acetate", Deardorff, D. R., Windham, C. Q. and Craney, C. L., Org. Synth. Coll. Vol. IX, 1998, 487 - 493.

Likewise, there are few instances in which a dynamic kinetic resolution 25 can be employed to give a maximum theoretical yield of 100% of a desired specified enantiomer, via equilibration of the enantiomers during the resolution. An example of this rare type of chemical dynamic kinetic resolution can be found in Reider, P. J., Davis, P., Hughes, D. L. and Grabowski, E. J., J. Org. Chem., 1987, 52, 955. An example of a rare alpha-substituted ketone 30 reductive dynamic kinetic resolution leading largely to a single diastereoisomer is described in Yamada, S., Mori, Y., Morimatsu, K., Ishizu, Y., Ozaki, Y., Yoshioka, R., Nakatani, T., and Seko, H., J. Org. Chem., 1996, 61, 8586. True enzyme-catalyzed diastereomeric dynamic kinetic resolutions are extremely rare for the preparation of single pure

diastereoisomers (compounds containing two chiral centers), since both chiral centers must be capable of equilibration. In this special case of enzyme-catalyzed diastereoisomer dynamic kinetic resolutions, only one of four possible chiral diastereoisomers is formed.

5

SUMMARY OF THE INVENTION

There exists a need for a reaction to produce (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol from its two chiral centers containing racemate in a greater than 50% yield as is typical in a simple chemical resolution process. There especially exists the need to produce this compound in a yield approximating 100%, that is, greater than 60% yield, preferably greater than 75% yield. Therefore, according to the present invention, there is provided an enzyme-catalyzed dynamic kinetic resolution method of preparing the compound (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol and/or desired salt forms from the corresponding racemate, which racemate will be referred to herein as (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol. There also exists the need to produce (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol and/or desired salt forms in higher yield in a commercial process that is inexpensive and environmentally feasible.

When the present invention is compared with prior methods of isolation, it will be apparent that according to the present invention, there will be a much higher yield (greater than 50% yield and generally greater than 80% yield) and there will be no need to isolate a chiral acid salt of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol from the mixture of diastereomeric salts. The present invention achieves these superior results by performing an enzyme-catalyzed dynamic kinetic resolution in a range of acidic to mildly basic pH's, namely pH 1 to pH 8. The schematic diagram herein illustrates how the undesired 2R, 3R-morpholinol (co-equal structures 3 and 4) is allowed to enzymatically "unravel" to the undesired 2R-hydroxybupropion (intermediate D), then to be enzymatically racemized or

equilibrated (intermediate D → intermediate C → intermediate B) → intermediate A) with the desired 2S-hydroxybupropion (intermediate A), which then enzymatically "ravels" back up to the desired 2S,3S- morpholinol (co-equal structures 1 and 2) which crystallizes out as a solid free base, or solid acid salt, or mixture thereof. This method will produce a desired free base or desired acid salt or mixture thereof in 70-100% yield, preferably 80-100% yield, with little or no mother liquor left over which is beneficial to the environment and/or eliminates further processing of the mother liquor before disposing of it. A chiral acid resolving agent is not needed in this process.

10 The absence of a chiral acid resolving agent further reduces manufacturing costs.

In short, there is provided a process for preparing (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol in its free base, its salt form, or both, which process comprises an enzyme-catalyzed dynamic kinetic resolution by equilibrating the two chiral centers of (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol.

To summarize, the present invention provides a process for making (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol which process comprises:

- 20 (1) dissolving (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol in a solvent and water and adjusting the pH to 1-8;
- (2) adding a catalytic amount of an esterase enzyme or a lipase enzyme optionally with stirring;
- (3) adding seed crystal selected from the group consisting of (i) (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base, (ii) a salt of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol, and (iii) a mixture of said free base and said salt of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol, while maintaining the pH between 1 and 8 and the reaction temperature between 10 C° and 50°C;
- 25 (4) quenching the reaction with an organic solvent and a base;
- (5) removing said esterase enzyme or said lipase enzyme from the reaction; and

(6) isolating (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base.

In a preferred embodiment, the present invention provides a process for making (+)-(2S,3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol in which the free base and/or salt form is further purified and/or "polished", thereby making it more acceptable for animal, especially human consumption. This preferred process comprises:

(1) dissolving (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol in a solvent and water and adjusting the pH to 1-8;

10 (2) adding a catalytic amount of an esterase enzyme or a lipase enzyme optionally with stirring;

(3) adding seed crystal selected from the group consisting of (i) (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base, (ii) a salt of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol, and (iii) a mixture of said free base and said salt of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol, while maintaining the pH between 1 and 8 and the reaction temperature between 10 C° and 50°C;

(4) quenching the reaction with an organic solvent and a base;

20 (5) removing said esterase enzyme or said lipase enzyme from the reaction;

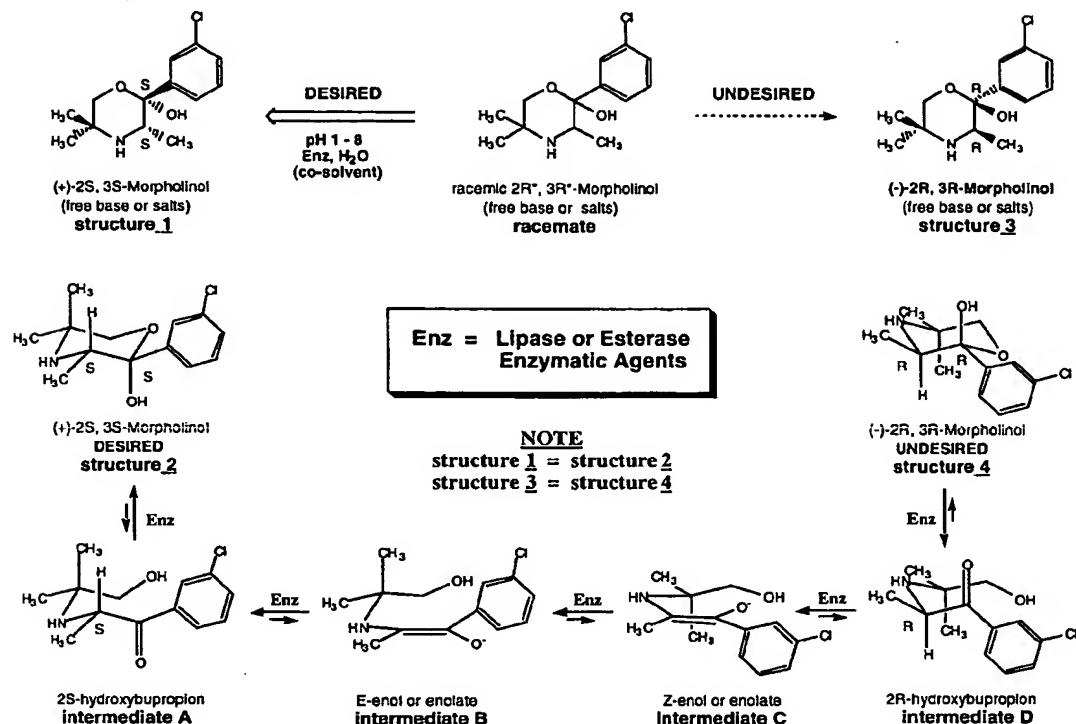
(6) isolating (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base;

25 (7) converting the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base into (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt; and

(8) recrystallizing said salt to produce a purer form of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt.

The schematic diagram set forth below illustrates the present enzyme-catalyzed or enzymatic dynamic kinetic resolution method for morpholinols:

ENZYMIC DYNAMIC KINETIC RESOLUTION OF MORPHOLINOLS



DETAILED DESCRIPTION OF THE INVENTION

5 1. Introduction

The present invention provides a method for making (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol, a single diastereoisomer from a two-chiral center racemate. The process is an unusual example of an "enzyme-catalyzed" or "enzyme-induced" asymmetric transformation, also termed a "second-order asymmetric transformation", but, importantly with two chiral centers equilibrating. (For one chiral center equilibrating asymmetric transformations see "Crystallization-Induced Asymmetric Transformations" by Jacques, J., Collet, A. and Wilen, S. H., in Enantiomers, Racemates and Resolutions (Krieger Publishing Company, Malabar, FL), 1991, Chapter 6, pp. 369-377. These processes are also referred to as a dynamic kinetic resolutions as disclosed in "Enantioselective Synthesis: The Optimum Solution", Partridge, J. J. and Bray, B. L. in Process Chemistry in the

Pharmaceutical Industry, (Gadamasetti, K. G., Ed.) Marcel Dekker, New York, NY, 1999, pp. 314-315.

In the process of the invention the following steps are performed:

(1) dissolving (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-

5 morpholinol in a solvent and water and adjusting the pH to 1-8;

(2) adding a catalytic amount of an esterase enzyme or a lipase enzyme optionally with stirring;

(3) adding seed crystal selected from the group consisting of (i) (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base, (ii) a salt 10 of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol, and (iii) a mixture of said free base and said salt of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol, while maintaining the pH between 1 and 8 and the reaction temperature between 10 C° and 50°C;

(4) quenching the reaction with an organic solvent and a base;

15 (5) removing said esterase enzyme or said lipase enzyme from the reaction; and

(6) isolating (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base.

The following additional steps are performed in a preferred

20 embodiment:

(7) converting the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base into (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt; and

25 (8) recrystallizing said salt to produce a purer form of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt.

2. Enzyme-Catalyzed Dynamic Kinetic Resolution Process to Prepare (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol and Its Pharmaceutically Acceptable Salts

30

St p On : Dissolving (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol in a solvent and water and adjusting the pH to 1-8.

In this step, (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol is dissolved in a solvent and water. The amount of water employed is at least 1% to hydrate the enzyme active site. Typically, the amount of water employed is between 1 - 25% based upon the solubility of 5 the racemic (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol and the insolubility of the desired (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base or desired (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol acid salt.

Suitable solvents include protic solvents such as alcohols such as 10 methanol and ethanol; ketones such as acetone; ethers such as methyl t-butyl ether; cyclic ethers such as tetrahydrofuran; nitriles such as acetonitrile; amides such as dimethylformamide; sulfoxides such as dimethyl sulfoxide, and the like. Typical concentrations of the racemate morpholinol in a given solvent or solvent combination are about 0.01 molar to about 2.0 molar. The 15 type and amount of solvent should be selected so as to completely or substantially completely dissolve the (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol. Use of the above-described types and amount of solvent allows the enzymatic kinetic resolution to take place efficiently leading to lower yields of the desired chiral end-product: (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and/or acid salts.

The pH is adjusted such that it is within the range of pH 1 to 8. The dissolved (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol is treated with an acid, a base, or a combination of acid and base as needed to adjust the pH of the solution to a pH of 1 to 8.

25 Suitable acids include, but are not limited to, inorganic acids (e.g., hydrochloric acid, sulfuric acid and phosphoric acid) and organic acids (e.g., acetic acid, etc).

Suitable bases include, but are not limited to, tertiary amine bases such 30 as trimethylamine and triethylamine; aromatic bases such as pyridine; inorganic bases such as ammonium hydroxide, sodium bicarbonate, ammonium carbonate, sodium carbonate and sodium hydroxide. The dissolved racemate to acid ratio would be about 100:1 to 1:2.

The temperature of the reaction solution can vary from 10 °C to 50°C and will vary depending on the particular esterase enzyme or lipase enzyme that is employed in a given process. Temperature is maintained by the application of heat or cooling using means known to those skilled in the 5 chemical arts.

Step Two: Adding a catalytic amount of an esterase enzyme or a lipase enzyme optionally with stirring.

Preferably, the esterase enzyme or lipase enzyme is employed in an 10 amount ranging from about 0.01 grams to 10 grams of enzyme per gram molecular weight of the racemic substrate.

The use of esterase enzyme or lipase enzyme is illustrated in the schematic diagram above. Esterase enzyme or lipase enzyme allows the undesired 2R, 3R-morpholinol (co-equal structures 3 and 4) to enzymatically 15 "unravel" to the undesired 2R-hydroxybupropion (intermediate D), then to be enzymatically racemized or equilibrated (intermediate D → intermediate C → intermediate B) → intermediate A) with the desired 2S-hydroxybupropion (intermediate A), which then enzymatically "ravels" back up to the desired 2S, 3S -morpholinol (co-equal structures 1 and 2) which crystallizes out as a solid 20 free base or solid acid salt or mixtures thereof. This method will produce a desired free base or solid acid salt in 80-100% yield, preferably 90-100% yield, with little or no mother liquor left over. A chiral acid resolving agent is not needed in this process.

As used herein, an amine-containing compound is commonly termed 25 an amine free base or "free base" if the amine exists in a non-protonated or non-salt form.

Examples of esterase enzymes and lipase enzymes useful in the process of the invention are shown in Tables 1 and 2, respectively.

**TABLE 1: EXAMPLES OF ESTERASE ENZYMES
USEFUL IN THE PROCESS OF THE INVENTION**

5 HORSE LIVER ESTERASE [EC3.1.1.1], from **horse liver**, (Aldrich Chemical Company or Sigma Chemical Company)

10 HUMAN LIVER ESTERASE [EC3.1.1.1], from **human liver**, (Aldrich Chemical Company or Sigma Chemical Company)

15 PIG LIVER ESTERASE [EC3.1.1.1], from **porcine liver (PLE)**, Sigma Chemical Company, E 3019

20 PIG LIVER ESTERASE ISOENZYME 1[EC3.1.1.1], from **porcine liver (PLE)**, Biochemika, Sigma Chemical Company, E 3019

25 RABBIT LIVER ESTERASE [EC3.1.1.1], from **rabbit liver**, Sigma Chemical Company, E 9636

30 ESTERASE [EC3.1.1.1] from **Bacillus sp.**, Biochemika (Aldrich Chemical Company or Sigma Chemical Company)

35 ESTERASE [EC3.1.1.1] from **Mucor miehei**, Biochemika (Aldrich Chemical Company or Sigma Chemical Company)

40 ESTERASE [EC3.1.1.1] from **Rhizopus oryzae**, Biochemika (Aldrich Chemical Company or Sigma Chemical Company)

45 ESTERASE [EC3.1.1.1] from **Rhizomucor miehei**, Biochemika (Aldrich Chemical Company or Sigma Chemical Company)

ESTERASE [EC3.1.1.1] from **Saccharomyces cerevisiae**, Biochemika (Aldrich Chemical Company or Sigma Chemical Company)

ESTERASE [EC3.1.1.1] from **Streptomyces diastalochromgenes**, Biochemika (Aldrich Chemical Company or Sigma Chemical Company)

ESTERASE [EC3.1.1.1] from **Stearotherophilus**, Biochemika (Aldrich Chemical Company or Sigma Chemical Company)

ESTERASE [EC3.1.1.1] from **Thermoanaerobium brockii**, Biochemika (Aldrich Chemical Company or Sigma Chemical Company)

ESTERASE [EC3.1.1.1] from **Thermomyces lanuginosus**, Biochemika (Aldrich Chemical Company or Sigma Chemical Company)

**TABLE 2: EXAMPLES OF LIPASE ENZYMES USEFUL
IN THE PROCESS OF THE INVENTION**

5 LIPASE [EC3.1.1.3] from **Aspergillus oryzae**, Biochemika (Aldrich Chemical Company, 62285)

10 LIPASE [EC3.1.1.3] from **Candida antarctica**, Biochemika (Aldrich Chemical Company, 62299)

15 LIPASE [EC3.1.1.3] from **Candida cylindracea**, Biochemika (Aldrich Chemical Company, 62302)

LIPASE [EC3.1.1.3] from **Candida utilis**, Biochemika (Aldrich Chemical Company, 62307)

15 LIPASE [EC3.1.1.3] from **Mucor javanicus**, Biochemika (Aldrich Chemical Company, 62304)

LIPASE [EC3.1.1.3] from **Mucor miehei**, Biochemika (Aldrich Chemical Company, 62298)

20 LIPASE from **Pseudomonas fluorescens**, (Aldrich Chemical Company, 39,044-5)

LIPASE from **Pseudomonas fluorescens**, **P-30 Amano** (Amano Enzyme Company, P-30)

25 LIPASE from **Pseudomonas fluorescens**, **P-800 Amano** (Amano Enzyme Company, P-800)

LIPASE [EC3.1.1.3] from **Rhizomucor arrhizus**, Biochemika (Aldrich Chemical Company, 62305)

30 LIPASE [EC3.1.1.3] from **Rhizomucor miehei**, Biochemika (Aldrich Chemical Company, 62291)

LIPASE [EC3.1.1.3] from **Thermus flavus**, Biochemika (Aldrich Chemical Company, 62295)

35 LIPASE [EC3.1.1.3] from **Thermus thermophilus**, Biochemika (Aldrich Chemical Company, 62296)

LIPASE, IMMOBILIZED in Sol-Gel-AK [EC3.1.1.3] from **Aspergillus niger**, Biochemika (Aldrich Chemical Company, 62281)

40 LIPASE, IMMOBILIZED in Sol-Gel-AK [EC3.1.1.3] from **Mucor miehei**, Biochemika (Aldrich Chemical Company, 62282)

LIPASE, IMMOBILIZED in Sol-Gel-AK [EC3.1.1.3] from **Pseudomonas fluorescens**, Biochemika (Aldrich Chemical Company, 62283)

45 LIPASE, IMMOBILIZED in Sol-Gel-AK from **Candida antarctica**, Biochemika (Aldrich Chemical Company, 62277)

50 LIPASE, IMMOBILIZED in Sol-Gel-AK from **Candida cylindracea**, Biochemika (Aldrich Chemical Company, 62278)

LIPASE, IMMOBILIZED in Sol-Gel-AK from **Pseudomonas cepacia**, Biochemika (Aldrich Chemical Company, 62279)

55 LIPASE B, recombinant [EC3.1.1.3] from **Candida antarctica**, Biochemika (Aldrich Chemical Company, 62288)

LIPASE, PORCINE PANCREAS (PPL) from **Porcine Pancreas**, Sigma Chemical Company, L 0382

5 LIPASE, HUMAN PANCREAS (HPL) from **Human Pancreas**, Sigma Chemical Company, L 9780

As necessary the pH of the solution is maintained within a pH range of
10 1 to 8 with acid, base, or combination thereof as previously set forth in step 1
above. A pH within this range is desired, and will vary within the disclosed pH
1-8 range depending upon the esterase or lipase chosen for each enzyme-
catalyzed dynamic kinetic resolution reaction. Stirring when employed is
accomplished by means well-known to those skilled in the art.

15

Step Three: Adding seed crystal selected from the group consisting
of (i) (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base,
(ii) a salt of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol, and
(iii) a mixture of said free base and said salt of (+)-(2S, 3S)-2-(3-
20 chlorophenyl)-3,5,5-trimethyl-2-morpholinol, while maintaining the pH between
1 and 8 and the reaction temperature between 10 C° and 50°C.

The amount of seed crystal added includes, but is not limited to about
10 mg (for small lab scale reactions of about 10 mL total reaction volume) to
about 1.0 gram (for large scale reactions of about a liter to multi-liter total
25 reaction volumes) with stirring at about 10 -100 revolutions per minute (rpms).

If the stirring is too fast, the enzyme may degrade due to shearing
forces on the enzyme protein. If the stirring is too slow, crystallization may not
be uniform or may occur too quickly leading to a large amount of solid in a ball
or clump. This is undesirable and may also cause the stirring shaft to break
30 or the stirring shaft motor to overheat.

The stirring may be provided by any means, for instance, by
mechanical stirring with one of a number of types of paddle stirrers being
suitable. Plastic paddles or metal paddles without sharp edges are preferred.

The specific enzyme chosen, the pH and the temperature chosen will
35 optimize each enzyme-catalyzed dynamic kinetic resolution reaction. In this

step, the pH is maintained between about pH 1 to about 8, varying depending upon the particular enzyme that is employed in a given reaction. Again, the pH is important in stabilizing the esterase or lipase protein enzyme while performing the enzymatically-catalyzed dynamic kinetic resolution. The

5 combination of esterase or lipase as illustrated in the schematic diagram (above) allows the undesired 2R, 3R-morpholinol (co-equal structures 3 and 4) to enzymatically "unravel" to the undesired 2R-hydroxybupropion (intermediate D), then to be enzymatically racemized or equilibrated (intermediate D → intermediate C → intermediate B) → intermediate A) with
10 the desired 2S-hydroxybupropion (intermediate A), which then enzymatically "ravels" back up to the desired 2S, 3S -morpholinol (co-equal structures 1 and 2) which crystallizes out as a solid free base or solid acid salt or a mixture thereof. This method will produce a desired free base or solid acid salt in 80-100% yield, preferably 90-100% yield, with little or no mother liquor left over.
15 A chiral acid resolving agent is not needed in this process.

If the pH is too high, (pH 12-14), the esterase or lipase may degrade and denature. At pH 12-14 these conditions may also degrade the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base to a number of by-products, including the degradant - the basic salt of meta-chlorobenzoic
20 acid (i.e. meta-chlorobenzoic acid, sodium salt, as an example).

If the pH is too low, (pH <1), there may be no equilibration of the (-)-(2R, 3R)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol to the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol via the 2R- and 2S-hydroxybupropion molecules. This important equilibration step is both pH and
25 enzyme dependent (see schematic diagram above).

Additional acid, base, or both may be added to maintain the desired pH. Any of the above-listed acids or bases are suitable. However, preferably the same acid or base is used throughout the present enzyme-catalyzed dynamic kinetic resolution process.

30

Step Four: Quenching the reaction with an organic solvent and a base.

In this step, the solid mixture of esterase enzyme or lipase enzyme and desired reaction product (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base or acid salt thereof such as, for example, (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt or 5 hydrogen sulfate salt is "partitioned" between an organic phase (solvent) phase and basic aqueous phase (base).

In this step, any crystalline acid salts that are present are converted into the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base. This may be accomplished by making the aqueous base solution from a 10 strong base such as ammonium hydroxide, potassium hydroxide or sodium hydroxide in water to pH >10. In this aqueous phase, the pH is basic enough to convert all of the acids to their basic water-soluble salts (i. e., for example, to an ammonium salt or sodium salt).

The organic phase is made up of an organic solvent, e. g., methylene 15 chloride, ethyl acetate, methyl t-butyl ether, and the like. The type and amount of organic solvent should be selected to completely or substantially completely extract the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base into the organic phase.

20 **Step Five:** Removing said esterase enzyme or said lipase enzyme from the reaction.

With the desired free base form of the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol in the organic phase and the acid salt form of the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol in the 25 aqueous phase, filtration through a sintered glass filter funnel, a Gooch filter, a pan filter, or a Rosemund filter will remove/recover the insoluble esterase or lipase enzyme protein which is insoluble in both the organic and aqueous phases. The removed/recovered esterase or lipase enzyme then may be recycled and re-used in future enzymatically controlled dynamic kinetic 30 resolution reactions. The enzyme is washed with additional organic phase solvent and water and stored as a wet cake at ambient temperature or refrigerated temperatures for future re-use.

Step Six: Isolation of the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base.

The organic phase from Step Four contains (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base. Additional free base can be isolated from the filtered aqueous phase by extraction with the organic solvent, e. g., methylene chloride, ethyl acetate, methyl t-butyl ether, and the like.

The type and amount of organic solvent should be selected so as to completely, or substantially completely, extract the free base into the organic phase. If not enough organic solvent is used, not all of the free base will be extracted from the aqueous phase into the organic phase. If too much organic solvent is used, the final evaporation of the organic phase will take longer than necessary. The combined organic phases are separated from the aqueous phase via phase partitioning by methods known in the art.

Evaporation of the organic phase then yields the desired (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base.

Step Seven: Conversion of the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base into (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol salt.

This step may be performed by addition of more than one equivalent of an inorganic acid (e.g., hydrochloric acid) and a co-solvent (or, alternatively, by addition of more than one equivalent of hydrogen chloride gas and a co-solvent), such that the pH of the solution or mixture reaches pH 1-2. The amount of either acid (e.g., hydrochloric acid) or gas (e.g., hydrogen chloride) should be selected so as to completely, or substantially completely, convert the free base into the salt (e.g., hydrochloride salt form). If not enough of either the hydrochloric acid or hydrogen chloride gas is used, the conversion will be incomplete, and the yield will, therefore, be reduced. If too much of either the hydrochloric acid or hydrogen chloride is used, there should be no problem other than excess waste generation.

Also, the type and amount of co-solvent should be selected to aid in the dissolving of the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and/or to aid in the precipitating of the desired final product (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol

5 hydrochloride salt. Amounts and ranges of hydrogen chloride or hydrochloric acid and solvents are at least 1 equivalent of hydrogen chloride in an organic solvent or 1 equivalent of hydrochloric acid (i.e., hydrogen chloride in aqueous solvent). Suitable solvents include methanol, ethanol, ethyl acetate, isopropyl acetate, acetonitrile, and the like. Suitable co-solvents can include ethers
10 such as diethyl ether, methyl tert-butyl ether, diphenyl ether; aromatic hydrocarbons such as benzene or toluene; and/or aliphatic hydrocarbons such as hexane or heptane.

For example, (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt is dissolved in methanol, filtered and the co-15 solvent ethyl acetate is added. Under vacuum, sufficient methanol is removed so that the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt crystallizes from a solution comprising mainly ethyl acetate (about 50% to 100% of the original solvent volume).

20 The amount of solvent present should be enough to prepare 0.1 molar to 4.0 molar solutions. Typically the co-solvent is present in an amount of 10% to 100% of the solvent volume.

Step Eight: Recrystallizing the salt of step 7 to produce a purer form of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol
25 hydrochloride salt.

A final recrystallization is performed by conducting a polishing filtration, followed by a crystallization with one or more organic solvents or solvent combinations. To carry out a "final crystallization" of a drug substance to meet regulatory guidelines and regulations in the great majority of cases, it is
30 necessary to fully dissolve the material to be crystallized, and then filter this solution.

Termed a "polishing filtration", this serves to remove trace amounts of extraneous material (e. g., dust, paper, and cloth fibers that may be present in small amounts), prior to the final crystallization. Accordingly, the amount of solvent present must be sufficient to dissolve all of the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt. If too little solvent is used, it will not be possible to dissolve all of the hydrochloride salt and accomplish this needed recrystallization procedure including the "polishing filtration purification". Too much solvent will result in lower yields of final crystalline product (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt.

Suitable organic solvents include methanol, ethanol, ethyl acetate, isopropyl acetate, acetonitrile, and the like, or solvent mixtures thereof. The initial concentration of the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt in the organic solvent or solvent mixture ranges from 0.1 molar to 4.0 molar and is capable of being filtered to remove insoluble impurities such as dust and related particulate matter.

EXAMPLES

20 **Experiment 01a: Preparation of Chiral Free Base Using a Lipase Enzyme**

A total of 255.8 gm (1.0 mole) of (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol is dissolved in 2.0 liters of acetone containing 1% water and stirred at room temperature and enough 0.1 N hydrochloric acid is added to bring the pH of this solution to pH 6-7.

25 As this solution is slowly mechanically stirred, portions totaling 5.0 gm of lipase from *Pseudomonas fluorescens*, P-30 Amano (Amano Enzyme Company, P-30) is added along with 0.1 N hydrochloric acid and 0.1 N sodium hydroxide to maintain this now heterogeneous mixture at pH 6 to pH 7. At this point the heterogenous solution is seeded with 0.5 gm each of (+)-(2S, 30) 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and its hydrochloride salt. The mixture is stirred for 16 hours to aid the precipitation

of desired solid forms of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and its hydrochloride salt from the solution.

The reaction mixture containing (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and its hydrochloride salt is partitioned into
5 an organic phase and an aqueous phase after stirring with a 1:1 mixture of 2.5 liters of methylene chloride and 2.5 liters of de-ionized water containing 3.0-3.5 equivalents of aqueous ammonium hydroxide base (pH >10). The two phases containing insoluble lipase protein at the interface are filtered to remove the lipase enzyme protein. An additional 1:1 mixture of 500 ml of
10 methylene chloride and 500 ml of de-ionized water are stirred with the collected lipase enzyme protein and filtered. The insoluble filter cake of recovered lipase enzyme protein is bottled and stored in a refrigerator at 4-10 C° for future re-use.

The organic phase of the combined filtrates is then separated from the
15 aqueous phase of the combined filtrates . The separated aqueous phase is extracted with an additional 1.0 liter of methylene chloride. This organic phase resulting therefrom is combined with the previous organic phase. The aqueous phase is then discarded. The combined organic phases are washed with 1 liter of de-ionized water and this washed organic phase is then
20 separated from the aqueous wash and concentrated under vacuum to yield crude (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base.

Experiment 01b: Preparation of Chiral Hydrochloride Salt

A total of 200 gm of crude (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base is dissolved in 1 liter of ethyl acetate with heating as necessary. A 5-6 molar solution of hydrochloric acid in methanol is added until a white precipitate forms and the pH of the solution reaches and is maintained at about pH 1-2. The mixture is then stirred for one hour and cooled to 10° C for one additional hour with stirring. The desired (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt is isolated
30 by filtration, washed with 49:1 ethyl acetate-methanol and dried under vacuum. In this manner up to 97% enantiomeric excess (97% ee) pure

quality (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is prepared.

Experiment 01c: Polishing Filtration and Crystallization of Chiral

5 Hydrochloride Salt

The (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is subjected to a polishing filtration and crystallization. This salt, from Experiment 1b, is dissolved in sufficient methanol to give a homogeneous solution which is filtered to remove any inert particulate matter 10 using a sintered glass filter funnel. The filtered solution is diluted with 1 - 3 volumes of ethyl acetate and concentrated under reduced pressure to selectively remove some of the methanol and to induce crystallization. This now heterogeneous solution is stirred for 2 - 4 hours at ambient temperature to complete the crystallization process. The solid mass of desired product is 15 then collected by filtration and dried. In this manner 99+% enantiomeric excess (99+% ee) pure quality (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is prepared.

Experiment 02a: Preparation of Chiral Free Base Using a Lipase Enzyme

20 A total of 255.8 gm (1.0 mole) of (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol is dissolved in 2.0 liters of acetone containing 1% water and stirred at room temperature and enough 0.1 N hydrochloric acid is added to bring the pH of this solution to pH 6-7.

25 As this solution is slowly mechanically stirred, portions totaling 2.0 gm of lipase from *Pseudomonas fluorescens*, P-800 Amano (Amano Enzyme Company, P-800) was added along with 0.1 N hydrochloric acid and 0.1 N sodium hydroxide to maintain this now heterogeneous mixture at pH 6 to pH7. At this point the heterogeneous solution is seeded with 0.5 gm each of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and its 30 hydrochloride salt. The mixture is stirred for 16 hours to aid the precipitation of desired solid forms of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and its hydrochloride salt from the solution.

The reaction mixture containing (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and its hydrochloride salt is partitioned into an organic phase and an aqueous phase after stirring with a 1:1 mixture of 2.5 liters of methylene chloride and 2.5 liters of de-ionized water containing 5 3.0-3.5 equivalents of aqueous ammonium hydroxide base (pH >10). The two phases containing insoluble lipase protein at the interface are filtered to remove the lipase enzyme protein. An additional 1:1 mixture of 500 ml of methylene chloride and 500 ml of de-ionized water are stirred with the collected lipase enzyme protein and filtered. The insoluble filter cake of 10 recovered lipase enzyme protein is bottled and stored in a refrigerator at 4-10 C° for future re-use.

The organic phase of the combined filtrates is then separated from the aqueous phase of the combined filtrates. The separated aqueous phase is extracted with an additional 1.0 liter of methylene chloride. This organic 15 phase resulting therefrom is combined with the previous organic phase. The aqueous phase is then discarded. The combined organic phases are washed with 1 liter of de-ionized water and this washed organic phase is then separated from the aqueous wash and concentrated under vacuum to yield crude (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base.

20

Experiment 02b: Preparation of Chiral Hydrochloride Salt

A total of 200 gm of crude (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base is dissolved in 1 liter of ethyl acetate with heating as necessary. A 5-6 molar solution of hydrochloric acid in methanol is 25 added until a white precipitate forms and the pH of the solution reaches and is maintained at about pH 1-2. The mixture is then stirred for one hour and cooled to 10° C for one additional hour with stirring. The desired (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt is isolated by filtration, washed with 49:1 ethyl acetate-methanol and dried under 30 vacuum. In this manner up to 98% enantiomeric excess (98% ee) pure quality (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is prepared.

Experiment 02c: Polishing Filtration and Crystallization of Chiral Hydrochloride Salt

The (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol

5 hydrochloride is subjected to a polishing filtration and crystallization. This salt, from Experiment 2b, is dissolved in sufficient methanol to give a homogeneous solution which is filtered to remove any inert particulate matter using either a sintered glass filter funnel. The filtered solution is diluted with 1-3 volumes of ethyl acetate and concentrated under reduced pressure to

10 selectively remove some of the methanol and to induce crystallization. This now heterogeneous solution is stirred for 2 - 4 hours at ambient temperature to complete the crystallization process. The solid mass of desired product is then collected by filtration and dried. In this manner 99+% enantiomeric excess (99+% ee) pure quality (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is prepared.

15

Experiment 03a: Preparation of Chiral Free Base Using an Esterase Enzyme

A total of 255.8 gm (1.0 mole) of (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-

20 3,5,5-trimethyl-2-morpholinol is dissolved in 2.0 liters of acetone containing 1% water and stirred at room temperature and enough 0.1 N sulfuric acid is added to bring the pH of this solution to pH 5 - pH 6.

25 As this solution is slowly mechanically stirred, portions of esterase [EC3.1.1.1] from Thermoanaerobium brockii, Biochemika (Aldrich Chemical Company) is added along with 0.1 N sulfuric acid and 0.1 N sodium hydroxide to maintain this now heterogeneous mixture at pH 5 to pH 6. At this point the heterogeneous solution is seeded with 0.5 gm each of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and hydrogen sulfate salt. The mixture is stirred for 16 hours to aid the precipitation of

30 desired solid forms of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and hydrogen sulfate salt from the solution.

The reaction mixture containing (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and hydrogen sulfate salt is partitioned into an organic phase and an aqueous phase after stirring with a 1:1 mixture of 5 2.5 liters of methylene chloride and 2.5 liters of de-ionized water containing 3.0-3.5 equivalents of aqueous ammonium hydroxide base (pH >10). The two phases containing insoluble esterase protein at the interface are filtered to remove the esterase enzyme protein. An additional 1:1 mixture of 500 ml of methylene chloride and 500 ml of de-ionized water are stirred with the 10 collected esterase enzyme protein and filtered. The insoluble filter cake of recovered esterase enzyme protein is bottled and stored in a refrigerator at 4-10 C° for future re-use.

The organic phase of the combined filtrates is then separated from the aqueous phase of the combined filtrates . The separated aqueous phase is 15 extracted with an additional 1.0 liter of methylene chloride. This organic phase resulting therefrom is combined with the previous organic phase. The aqueous phase is then discarded. The combined organic phases are washed with 1 liter of de-ionized water and this washed organic phase is then separated from the aqueous wash and concentrated under vacuum to yield 20 crude (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base.

Experiment 03b: Preparation of Chiral Hydrochloride Salt

A total of 200 gm of crude (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base is dissolved in 1 liter of ethyl acetate with 25 heating as necessary. A 5-6 molar solution of hydrochloric acid in methanol is added until a white precipitate forms and the pH of the solution reaches and is maintained at about pH 1-2. The mixture is then stirred for one hour and cooled to 10° C for one additional hour with stirring. The desired (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt is isolated 30 by filtration, washed with 49:1 ethyl acetate-methanol and dried under vacuum. In this manner up to 97% enantiomeric excess (97% ee) pure

quality (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is prepared.

Experiment 03c: Polishing Filtration and Crystallization of Chiral Hydrochloride Salt

If required, the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is subjected to a polishing filtration and crystallization. This salt, from Experiment 3b, is dissolved in sufficient methanol to give a homogeneous solution which is filtered to remove any inert particulate matter using either a sintered glass filter funnel. The filtered solution is diluted with 1 - 3 volumes of ethyl acetate and concentrated under reduced pressure to selectively remove some of the methanol and to induce crystallization. This now heterogeneous solution is stirred for 2 - 4 hours at ambient temperature to complete the crystallization process. The solid mass of desired product is then collected by filtration and dried. In this manner 99+% enantiomeric excess (99+% ee) pure quality (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is prepared.

Experiment 04a: Preparation of Chiral Free Base Using an Esterase

20 Enzyme

A total of 255.8 gm (1.0 mole) of (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol is dissolved in 2.0 liters of acetone containing 1% water and stirred at room temperature as enough 0.1 N sulfuric acid is added to bring the pH of this solution to pH 5 - pH 6.

As this solution is slowly mechanically stirred, portions of esterase [EC3.1.1.1] from *Thermomyces lanugenosus*, Biochemika (Aldrich Chemical Company) was added along with 0.1 N sulfuric acid and 0.1 N sodium hydroxide to maintain this now heterogeneous mixture at pH 5 to pH 6. At this point the heterogeneous solution is seeded with 0.5 gm each of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and hydrogen sulfate salt. The mixture is stirred for 16 hours to aid the precipitation of

desired solid forms of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and hydrogen sulfate salt from the solution.

The reaction mixture containing (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base and hydrogen sulfate salt is partitioned into 5 an organic phase and an aqueous phase after stirring with a 1:1 mixture of 2.5 liters of methylene chloride and 2.5 liters of de-ionized water containing 3.0-3.5 equivalents of aqueous ammonium hydroxide base (pH >10). The two phases containing insoluble esterase enzyme enzyme protein at the interface are filtered to remove the esterase enzyme protein. An additional 1:1 mixture 10 of 500 ml of methylene chloride and 500 ml of de-ionized water are stirred with the collected esterase enzyme protein and filtered. The insoluble filter cake of recovered esterase enzyme protein is bottled and stored in a refrigerator at 4-10 C° for future re-use.

The organic phase of the combined filtrates is then separated from the 15 aqueous phase of the combined filtrates. The separated aqueous phase is extracted with an additional 1.0 liter of methylene chloride. This organic phase resulting therefrom is combined with the previous organic phase. The aqueous phase is then discarded. The combined organic phases are washed with 1 liter of de-ionized water and this washed organic phase is then 20 separated from the aqueous wash and concentrated under vacuum to yield crude (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base.

Experiment 04b: Preparation of Chiral Hydrochloride Salt

A total of 200 gm of crude (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base is dissolved in 1 liter of ethyl acetate with 25 heating as necessary. A 5-6 molar solution of hydrochloric acid in methanol is added until a white precipitate forms and the pH of the solution reaches and is maintained at about pH 1-2. The mixture is then stirred for one hour and cooled to 10° C for one additional hour with stirring. The desired (+)-(2S, 3S)-30 2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt is isolated by filtration, washed with 49:1 ethyl acetate-methanol and dried under vacuum. In this manner up to 97% enantiomeric excess (97% ee) pure

quality (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is prepared.

Experiment 04c: Polishing Filtration and Crystallization of Chiral Hydrochloride Salt

If required, the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is subjected to a polishing filtration and crystallization. This salt, from Experiment 4b, is dissolved in sufficient methanol to give a homogeneous solution which is filtered to remove any inert particulate matter using either a sintered glass filter funnel. The filtered solution is diluted with 1 - 3 volumes of ethyl acetate and concentrated under reduced pressure to selectively remove some of the methanol and to induce crystallization. This now heterogeneous solution is stirred for 2 - 4 hours at ambient temperature to complete the crystallization process. The solid mass of desired product is then collected by filtration and dried. In this manner 99+% enantiomeric excess (99+% ee) pure quality (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride is prepared.

All cited patents, publications, co-pending applications, and provisional applications referred to in this application are herein incorporated by reference.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

CLAIMS

What is claimed is:

1. A process for preparing (+)-(2S,3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol in its free base, its salt form, or both, which process comprises an enzyme-catalyzed dynamic kinetic resolution by equilibrating the two chiral centers of (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol.
2. The process according to claim 1 which process comprises:
 - (1) dissolving (+/-)-(2R*, 3R*)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol in a solvent and water and adjusting the pH to 1-8;
 - (2) adding a catalytic amount of an esterase enzyme or a lipase enzyme optionally with stirring and optionally adjusting the pH to 1-8;
 - (3) adding seed crystal selected from the group consisting of (i) (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base, (ii) a salt of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol, and (iii) a mixture of said free base and said salt of (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol, while maintaining the pH between 1 and 8 and the reaction temperature between 10 C° and 50°C;
 - (4) quenching the reaction with an organic solvent and a base;
 - (5) removing said esterase enzyme or said lipase enzyme from the reaction; and
 - (6) isolating (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base.

3. The process according to claim 2 further comprising the steps of converting the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol in its free base form into (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol its salt form; and recrystallizing said salt to produce a purer form of the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol salt.
4. The process according to claim 3 wherein the salt is (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt.
5. The process according to claim 2, wherein the enzyme is an esterase enzyme.
6. The process according to claim 2, wherein the enzyme is a lipase enzyme.
7. The process according to claim 5, wherein the esterase enzyme is esterase from *Thermoanaerobium brockii* or esterase from *Thermomyces lanugenosus*.
8. The process according to claim 6, wherein the lipase enzyme is lipase from *Pseudomonas fluorescens*.
9. The process according to claim 2, wherein said pH 1-8 is adjusted using (i) an acid, (ii) a base, or (iii) a combination of an acid and a base.
10. The process according to claim 9, wherein said acid is an organic or an inorganic acid; and said base is an organic or an inorganic base.

11. The process according to claim 10, wherein said acid is selected from the group consisting of hydrochloric acid, sulfuric acid, phosphoric acid, and acetic acid.
12. The process according to claim 10, wherein said base is selected from the group consisting of an alkali metal hydrogen carbonate, an alkali metal carbonate, an alkali metal hydroxide, ammonium hydroxide, an aliphatic amine, and an aromatic amine.
13. The process according to claim 2, wherein the solvent is selected from the group consisting of a protic solvent, a ketonic solvent, and an ether solvent.
14. The process according to claim 13, wherein said solvent is selected from the group consisting methanol, ethanol, acetone, and tetrahydrofuran.
15. The process according to claim 2, wherein the seed crystal is a (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt.
16. The process according to claim 2, wherein step (4) has a pH >10.
17. The process according to claim 2, further comprising recycling the esterase or lipase enzyme removed in step (5) into step (2).
18. The process according to claim 2, further comprising: isolating (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol free base in Step (5) such that it is substantially free from enzyme protein in Step (5).
19. The process according to claim 16, wherein said isolating is performed using a strong base in an excess amount.

20. The process according to claim 19, wherein said strong base is selected from the group consisting of ammonium hydroxide, potassium hydroxide, and sodium hydroxide in water.
21. The process according to claim 4, wherein said free base is converted into said hydrochloride salt by (i) addition of more than one equivalent of hydrochloric acid and a co-solvent or by (ii) addition of more than one equivalent of hydrogen chloride gas and a co-solvent, such that the pH is between 1-2.
22. The process according to claim 21, wherein said co-solvent is at least one selected from the group consisting of methanol, ethanol, ethyl acetate, isopropyl acetate, and acetonitrile.
23. The process according to claim 22, further comprising: recrystallizing the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt.
24. The process according to claim 23, wherein said recrystallizing is performed by polishing filtration and crystallization in the presence of at least one organic solvent.
25. The process according to claim 24, wherein said organic solvent is selected from the group consisting of methanol, ethanol, ethyl acetate, isopropyl acetate, and acetonitrile.

ABSTRACT OF THE DISCLOSURE

A process for preparing (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol and pharmaceutically acceptable salts such as the (+)-(2S, 3S)-2-(3-chlorophenyl)-3,5,5-trimethyl-2-morpholinol hydrochloride salt via
5 enzyme-catalyzed dynamic kinetic resolutions is provided.

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/034667

International filing date: 21 October 2004 (21.10.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/514,694
Filing date: 27 October 2003 (27.10.2003)

Date of receipt at the International Bureau: 13 December 2004 (13.12.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse